

***IN THE UNITED STATES PATENT AND TRADEMARK OFFICE***

Applicant: Neil Fraser Fairweather et al.  
Title: TETANUS TOXIN POLYPEPTIDES  
Appl. No.: 10/018,997  
Filing Date: December 26, 2001  
Examiner: Unassigned  
Art Unit: Unassigned

**SUBSEQUENT PRELIMINARY AMENDMENT**

Commissioner for Patents  
Box PCT  
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, Applicants respectfully request that the above-identified application be amended as follows:

**In the Specification:**

Please amend the Specification as shown:

Please delete the paragraph at page 21, lines 7-19 and replace it with the following paragraph:

*E. coli* strain BL21 ( $\lambda$ DE3, *ompT*, *hsdS<sub>B</sub>* (*r<sub>B</sub>-m<sub>B</sub>-1*), *gal*, *dcm*) was used as the host for the plasmids described below. Plasmid pKS1 contains a codon-optimised gene for the expression of the Hc fragment of TeNT under the control of the T7 promoter. It was created by PCR amplification (Pfu polymerase, Stratagene, Cambridge UK) of a 1357 bp fragment using pTETtac215 (Makoff *et al.*, 1989) as template and the oligonucleotides 5'GAGCATATGAAAAACCTTGAT (SEQ ID NO: 2) and 5'CGGATCCTTAGTCGTTGGTCCA (SEQ ID NO: 3) which introduce *Nde*I and *Bam*HI

sites at the 5' and 3' ends of the gene respectively. After blunt end ligation of the PCR product into the vector pCRScript (Stratagene) to form plasmid pJC6, the *Nde*I – *Bam*HI fragment was purified by agarose gel electrophoresis using a Qiaex II gel purification kit (Qiagen, West Sussex, UK), and subcloned into pET28a (Novagen, Cambridge UK) which has previously been digested with *Nde*I and *Bam*HI (Roche Molecular Biochemicals, East Sussex, UK). DNA manipulations were performed by standard procedures.

Please delete the TABLE 1 Mutants of TeNT Hc constructed at page 22, and replace it with the following TABLE:

**TABLE 1 Mutants of TeNT Hc constructed**

Mutant Name	Oligonucleotides used for site directed mutagenesis
M5	NF38: 5' to 3' GGTTGCGACTGGTACTTCTAAGGATCCGAATTG (SEQ ID. NO:4) NF41: 3' to 5' CGAATTCCGATCCTTAGAAGTACCGAGTCGAACC (SEQ ID. NO:5)
T1308A	NP49: 5' to 3' GACTGGTACTTCGTTCCGGCTGATGAAGGTTGGA (SEQ ID. NO:6) NP50: 3' to 5' GGTCCAACCTTCATCAGCCGAAACGAAGTACCAAG (SEQ ID. NO:7)
D1309A	NF51: 5' to 3' TGGTACTTCGTTCCGACCGCTGAAGGTTGGACGA (SEQ ID. NO:8) NF52: 3' to 5' CGTTGGTCCAACCTTCAGCGTCCGAACGAAGTA (SEQ ID. NO:9)
E1310A	NF57: 5' to 3' TACTTCGTTCCGACQATGCTGGTTGGACGCAACGAC (SEQ ID. NO:10) NF58: 3' to 5' GTCGTTGGTCCAACCAAGCATCGGTCGGAACGAAGTA (SEQ ID. NO:11)
M13	NF47: 5' to 3' TTCGTTCCGACCGATGAATAAGGATCCGAATTG (SEQ ID. NO:12) NF48: 3' to 5' CGAATTCCGATCCTTATTATCAGCGTCCGAACGAA (SEQ ID. NO:13)
M28	NF79: 5' to 3' GGTACCCACAACGTGTCAGCGAACCGTGACATCCTG (SEQ ID. NO:14) NF80: 5' to 3' CAGGATGTCACGGTCGGCTGACCGTTGTGGTACC (SEQ ID. NO:15)
M37	NF81: 5' to 3' CTGGGCTCTGGTTGGTACCAACGACCGAACCGTGAC (SEQ ID. NO:16) NF82: 5' to 3' GTCACGGTTCGGGTCGTTGCTACCAACCAGACCGAG (SEQ ID. NO:17)
M40	NF79: 5' to 3' GGTACCCACAAGGTCAAGCCGAACCGTGACATCCTG (SEQ ID. NO:18) NF80: 5' to 3' CAGGATGTCACGGTCGGCTGACCGTTGTGGTACC (SEQ ID. NO:19) and NF32: 5' to 3' CTTCTAACTGGTACTTCAACTCTCTGAAAGACAAAATCCTGGG (SEQ ID. NO:20) NF33: 3' to 5' CCCAGGATTTGTCTTCAGAGCGTTGAAGTACCAAGTTAGAAG (SEQ ID. NO:21)
M58	NF91: 5' to 3' GTTGGTTACCCGAAACTGCAGAACCTGGACAGAATT (SEQ ID. NO:22) NF92: 3' to 5' AATTCTGTCAGGTTCTGCAGTTCGGGTAACAAC (SEQ ID. NO:23)
M564	NF32: 5' to 3' CTTCTAACTGGTACTTCAACTCTCTGAAAGACAAAATCCTGGG (SEQ ID. NO:24) NF33: 3' to 5' CCCAGGATTTGTCTTCAGAGCGTTGAAGTACCAAGTTAGAAG (SEQ ID. NO:25)
M567	NF97: 5' to 3' CTAACGGTACTTCAACGCTCTGAAAGAGAAAATCCTGGG (SEQ ID. NO:26) NF98: 3' to 5' CCCAGGATTTGTCTTCAGAGCGTTGAAGTACCAAGTTAG (SEQ ID. NO:27)

Please delete the Amino acid sequence of *C. tetani* neurotoxin (TeNT) constructed at page 36, and replace it with the following TABLE:

Amino acid sequence of *C. tetani* neurotoxin (TeNT)

1	mpitinnfry	sdpvnndtii	mmeppycckgl	diyykafkit	driwivpervy	efgtkpedfn
61	ppssliegas	eyydpnylrt	dsdkdrflqt	mvklnfrikn	nvagealldk	iinaipylgn
121	syslldkfdt	nsnsvsfnll	eqdpsgattk	samltnliif	gpgpvlnkne	vrgivlrvdn
181	knyfpcrdgf	gsimqmafcp	eyvptfdnvi	enitsltigk	skyfqdpall	lmheihvh
241	glygmqvssh	eiipskqeiy	mqhtypisae	elftfggqda	nlisidiknd	lyekthdyk
301	aiannklsqvt	scndpnidid	sykqiyqqky	qfdkdsnggy	ivnedkfqil	ynsimygte
361	ielgkkfnik	trlsyfsmnh	dpvkipnlld	dtiyndtegf	nieskdlkse	ykgqnmrvnt
421	nafrnvdgsg	lvskliglck	kiipptnire	nlynrtaslt	dlggelciki	knedltfiae
481	knsfseepfq	deivsyntkn	kplnfnysld	kiivdynlqs	kitlpndrtt	pvtkgipyap
541	eyksnaasti	eibhiddnti	yqylyaqksp	ttlqritmtn	svddalinst	kiysyfpsvi
601	skvnqgaqgi	lflqwwrdii	ddftnessqk	ttidkisdv	tivpyigpal	nivkqgyegn
661	figalettgv	vllveyipei	tlpviaalsi	aesstqkeki	iktidnflek	ryekwievyk
721	lvkakwlgtv	ntqfqkrstyq	myrsleyqvq	aikkiidye	kiysgpdked	iadeinnlkn
781	kleekankam	ininifmres	srsflvngmi	neakkqllef	dtqsknilmq	yikanskfig
841	itelkkiesk	inkvfstpip	fsysknldcw	vdneedidvi	lkkstilnid	inndiisdis
901	gfnssvityp	daqlvpging	kaihlvnnes	sevivhkam	ieyndmfnnf	tvsfwlrvpk
961	vsashlegyg	tneysiissm	khhslsigsg	wsvsilkgnl	iwtkdsage	vrqitfrdlp
1021	dkfnaylank	wvfititndr	issanlying	vlmgsaeitg	lgaireddni	tlkldrennn
1081	nqyvsidkfr	ifckalnpke	ieklytsyls	itflrdfwgn	plyrdteyyl	ipvassskdv
1141	qlkahtdytny	ltnapsytnq	klniyyrrly	nglkfiikry	tpnneidsfv	ksgdfiklyv
1201	synnnehivg	ypkdgnafnn	ldxilrvgyn	apgipllykkm	eavklrdikt	ysvqlklydd
1261	knasglvgt	hngqigndpn	rdiliasnw	fnhlkdkig	cdwyfvptde	gwtnd

(SEQ ID NO: 1)